

What is claimed is:

*Sub A27*  
1. A method for identifying a modulator of quorum sensing signaling in bacteria, said method comprising:

providing a cell which comprises a quorum sensing controlled gene, wherein said cell is responsive to a quorum sensing signal molecule such that a detectable signal is generated;

10 10 contacting said cell with a quorum sensing signal molecule in the presence and absence of a test compound;

and detecting a change in the detectable signal to thereby identify said test compound as a modulator of quorum sensing signaling in bacteria.

15 15 2. The method of claim 1, wherein said cell further comprises means for generating said detectable signal.

20 20 3. The method of claim 2, wherein said signal generation means comprises a reporter gene, and wherein said quorum sensing signal molecule causes transcription of said reporter gene, said transcription providing said detectable signal.

25 25 4. The method of claim 3, wherein said reporter gene is operatively linked to a regulatory sequence of said quorum sensing controlled gene.

30 30 5. The method of claim 4, wherein said reporter gene is selected from the group consisting of *ADE1*, *ADE2*, *ADE3*, *ADE4*, *ADE5*, *ADE7*, *ADE8*, *ASP3*, *ARG1*, *ARG3*, *ARG4*, *ARG5*, *ARG6*, *ARG8*, *ARO2*, *ARO7*, *BARI*, *CAT*, *CHO1*, *CYS3*, *GAL1*, *GAL7*, *GAL10*, *GFP*, *HIS1*, *HIS3*, *HIS4*, *HIS5*, *HOM3*, *HOM6*, *ILV1*, *ILV2*, *ILV5*, *INO1*, *INO2*, *INO4*, *lacZ*, *LEU1*, *LEU2*, *LEU4*, *luciferase*, *LYS2*, *MAL*, *MEL*, *MET2*, *MET3*, *MET4*, *MET8*, *MET9*, *MET14*, *MET16*, *MET19*, *OLE1*, *PHO5*, *PRO1*, *PRO3*, *THR1*, *THR4*, *TRP1*, *TRP2*, *TRP3*, *TRP4*, *TRP5*, *URA1*, *URA2*, *URA3*, *URA4*, *URA5* and *URA10*.

35 35 6. The method of claim 5, wherein said reporter gene is *lacZ* or *GFP*.

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7. The method of claim 1, wherein said cell does not express said quorum sensing signal molecule.

8. The method of claim 7, wherein said quorum sensing signal molecule is produced by a second cell.

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9. ~~The method of claim 1, wherein said cell is a prokaryote or eukaryote.~~

10. The method of claim 9, wherein said cell is a bacterium.

11. The method of claim 8, wherein said second cell is a prokaryote or  
10 eukaryote.

12. The method of claim 11, wherein said second cell is a bacterium.

13. The method of claims 10 or 12, wherein said bacterium is a gram negative bacterium.

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14. The method of claim 13, wherein said gram negative bacterium is *Pseudomonas aeruginosa*.

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15. The method of claim 10, wherein said bacterium is a mutant strain of *Pseudomonas aeruginosa* which comprises a regulatory sequence of a quorum sensing controlled gene operatively linked to a reporter gene, wherein in said mutant strain, *lasI* and *rhII* are inactivated.

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16. The method of claim 12, wherein said second cell is wild type *Pseudomonas aeruginosa*.

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17. ~~The method of claim 1, wherein said quorum sensing controlled gene is endogenous to said cell.~~

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18. The method of claim 10, wherein said quorum sensing controlled gene encodes a virulence factor.

19. The method of claim 10, wherein said quorum sensing controlled gene encodes a polypeptide which inhibits a bacterial host defense mechanism.

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20. The method of claim 10, wherein said quorum sensing controlled gene encodes a polypeptide which regulates biofilm formation.

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21. The method of claim 1, wherein said quorum sensing signal molecule is an autoinducer of said quorum sensing controlled gene.

22. The method of claim 21, wherein said autoinducer is a homoserine lactone.

23. The method of claim 22, wherein said test compound is a homoserine lactone analog.

10 24. The method of claim 1, wherein said modulator inhibits an enzyme involved in the synthesis by said bacterium of said quorum sensing signal molecule.

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15 25. The method of claim 1, wherein said modulator inhibits reception of said quorum sensing signal molecule by said bacterium.

20 26. The method of claim 1, wherein said modulator scavenges said quorum sensing signal molecule.

27. A method for identifying a modulator of quorum sensing signaling in *Pseudomonas aeruginosa*, said method comprising:

providing a wild type strain of *Pseudomonas aeruginosa* which produces a quorum sensing signal molecule;

25 providing a mutant strain of *Pseudomonas aeruginosa* which comprises a reporter gene operatively linked to a regulatory sequence of a quorum sensing controlled gene, wherein said mutant strain is responsive to said quorum sensing signal molecule produced by said wild type strain, such that a detectable signal is generated;

30 contacting said mutant strain with said quorum sensing signal molecule and a test compound; and

detecting a change in the detectable signal to thereby identify said test compound as a modulator of quorum sensing signaling in *Pseudomonas aeruginosa*.

35 28. The method of claim 27, wherein in said mutant strain, *lasI* and *rhlII* are inactivated.

29. The method of claim 27, wherein said reporter gene is *lacZ* or *GFP*.

30. The method of claim 29, wherein said reporter gene is *lacZ*.

5 31. The method of claim 29, wherein said reporter gene is *GFP*.

32. The method of claim 31, wherein said reporter gene is a variant of *GFP*.

10 33. The method of claim 32, wherein said variant is GFPmut2.

34. The method of claim 27, wherein said mutant strain of *Pseudomonas aeruginosa* comprises a promoterless reporter gene inserted at a genetic locus in the chromosome of said *Pseudomonas aeruginosa*, wherein said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36.

25 35. The method of claim 34, wherein said promoterless reporter gene is inserted in said chromosome at a locus comprising a nucleotide sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:28 and SEQ ID NO:35.

30 36. The method of claim 34, wherein said reporter gene is contained in a transposable element.

37. A mutant strain of *Pseudomonas aeruginosa* comprising a promoterless reporter gene inserted at a genetic locus in the chromosome of said *Pseudomonas aeruginosa*, wherein said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36.

NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36.

5        38.      The mutant strain of claim 37, wherein said reporter gene is contained in a transposable element.

39.      The mutant strain of claim 37, wherein *lasI* and *rhII* are inactivated.

10       40.      The mutant strain of claim 37, wherein said strain is responsive to a quorum sensing signal molecule such that a detectable signal is generated by said reporter gene.

15       41.      The mutant strain of claim 37, wherein said reporter gene is *lacZ* or *GFP*.

42.      *The method of claim 41*, wherein said reporter gene is a variant of *GFP*.

43.      *The method of claim 42*, wherein said variant is GFPmut2.

20       44.      A method for identifying a modulator of a quorum sensing signaling in *Pseudomonas aeruginosa*, said method comprising:

25       providing a wild type strain of *Pseudomonas aeruginosa* which produces a quorum sensing signal molecule;

30       providing a mutant strain of *Pseudomonas aeruginosa* which comprises a promoterless reporter gene inserted at a genetic locus in the chromosome of said *Pseudomonas aeruginosa*, wherein said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36; and wherein said mutant strain is responsive to said quorum sensing signal molecule produced by said wild type strain, such that a detectable signal is generated by said reporter gene;

contacting said mutant strain with said quorum sensing signal molecule and a test compound; and

5        detecting a change in the detectable signal to thereby identify said test compound as a modulator of quorum sensing signaling in *Pseudomonas aeruginosa*.

45.      The method of claim 44, wherein said reporter gene is contained in a transposable element.

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46.      An isolated nucleic acid molecule comprising a nucleotide sequence, said nucleotide sequence comprising:

a regulatory sequence derived from the genome of *Pseudomonas aeruginosa*, wherein said regulatory sequence regulates a quorum sensing controlled genetic locus of the *Pseudomonas aeruginosa* chromosome, and wherein said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36; and

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a reporter gene operatively linked to said regulatory sequence.

47.      An isolated nucleic acid molecule comprising a quorum sensing controlled genetic locus derived from the genome of *Pseudomonas aeruginosa*, wherein said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36; and

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NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36, operatively linked to a reporter gene.

48. An isolated nucleic acid molecule comprising a polynucleotide having at least 80% identity to a quorum sensing controlled genetic locus derived from the genome of *Pseudomonas aeruginosa*, wherein said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36, operatively linked to a reporter gene.

49. An isolated nucleic acid molecule comprising a polynucleotide that hybridizes under stringent conditions to the complement of a nucleotide sequence comprising a quorum sensing controlled genetic locus derived from the genome of *Pseudomonas aeruginosa*, wherein said locus comprises a nucleotide sequence selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36, operatively linked to a reporter gene.

50. The nucleic acid molecule of any one of claims 46, 47, 48 and 49, wherein said reporter gene is contained in a transposable element.

51. A vector comprising the isolated nucleic acid molecule of any one of claims 46, 47, 48 and 49.

52. A cell containing an isolated nucleic acid molecule of any one of claims 46, 47, 48 and 49.

53. A method for identifying a modulator of quorum sensing signaling in bacteria, said method comprising:

providing the cell of claim 52, wherein said cell is responsive to a quorum sensing signal molecule such that a detectable signal is generated;

10 contacting said cell with a quorum sensing signal molecule in the presence and absence of a test compound;

and detecting a change in the detectable signal to thereby identify said test compound as a modulator of quorum sensing signaling in bacteria.

15 54. A compound which inhibits quorum sensing signaling in *Pseudomonas aeruginosa*, said compound having been identified by the method of claim 28.

20 55. The compound of claim 54, which inhibits quorum sensing signaling in *Pseudomonas aeruginosa* by inhibiting an enzyme involved in the synthesis of a quorum sensing signal molecule, by interfering with quorum sensing signal reception, or by scavenging the quorum sensing signal molecule.

25 56. A method for identifying a quorum sensing controlled gene in bacteria, said method comprising:

providing a cell which is responsive to a quorum sensing signal molecule such that expression of a quorum sensing controlled gene is modulated, and wherein modulation of the expression of said quorum sensing controlled gene generates a 30 detectable signal;

contacting said cell with a quorum sensing signal molecule;

and detecting a change in the detectable signal to thereby identify a quorum 35 sensing signaling controlled gene in bacteria.

57. The method of claim 56, wherein said cell further comprises means for generating said detectable signal.

58. The method of claim 57, wherein said signal generation means comprises a reporter gene, and wherein modulation of the expression of said quorum sensing controlled gene modulates transcription of said reporter gene, said transcription providing said detectable signal.

59. The method of claim 58, wherein said reporter gene is operatively linked to a regulatory sequence of said quorum sensing controlled gene.

10 60. The method of claim 58, wherein said reporter gene is operatively linked to said quorum sensing controlled gene.

61. The method of either of claims 59 and 60, wherein said reporter gene is contained in a transposable element.

15 62. The method of claim 58, wherein said reporter gene is selected from the group consisting of *ADE1*, *ADE2*, *ADE3*, *ADE4*, *ADE5*, *ADE7*, *ADE8*, *ASP3*, *ARG1*, *ARG3*, *ARG4*, *ARG5*, *ARG6*, *ARG8*, *ARO2*, *ARO7*, *BAR1*, *CAT*, *CHO1*, *CYS3*, *GAL1*, *GAL7*, *GAL10*, *GFP*, *HIS1*, *HIS3*, *HIS4*, *HIS5*, *HOM3*, *HOM6*, *ILV1*, *ILV2*, *ILV5*, *INO1*,  
20 *INO2*, *INO4*, *lacZ*, *LEU1*, *LEU2*, *LEU4*, *luciferase*, *LYS2*, *MAL*, *MEL*, *MET2*, *MET3*,  
*MET4*, *MET8*, *MET9*, *MET14*, *MET16*, *MET19*, *OLE1*, *PHO5*, *PRO1*, *PRO3*, *THR1*,  
*THR4*, *TRP1*, *TRP2*, *TRP3*, *TRP4*, *TRP5*, *URA1*, *URA2*, *URA3*, *URA4*, *URA5* and  
*URA10*.

25 63. The method of claim 56, wherein said quorum sensing signal molecule is produced by a second cell.

64. The method of claim 63, wherein said second cell is a prokaryote or eukaryote.

30 65. The method of claim 64, wherein said second cell is a bacterium.

66. The method of claim 56, wherein said cell is a prokaryote or eukaryote.

35 67. The method of claim 66, wherein said cell is a bacterium.

68. The method of either of claims 65 and 67, wherein said bacterium is a gram negative bacterium.

69. The method of claim 68, wherein said gram negative bacterium is *Pseudomonas aeruginosa*.

5 70. The method of claim 67, wherein said bacterium is a mutant strain of *Pseudomonas aeruginosa* in which *lasI* and *rhlI* are inactivated.

10 71. The method of claim 65, wherein said second cell is wild type *Pseudomonas aeruginosa*.

10 72. The method of claim 56, wherein said quorum sensing signal molecule is an autoinducer of said quorum sensing controlled gene.

15 73. The method of claim 72, wherein said autoinducer is a homoserine lactone, or an analog thereof.

74. The method of claim 56, wherein said quorum sensing signal molecule induces the expression of said quorum sensing controlled gene.

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